CELLULAR ORGANIZATION
Unit-2 D
Cell division and cell cycle

Batch- Morning

Duration : 1.5 Hrs.

Reference

- Cell and Molecular Biology: concepts and experiments by Gerald Karp
- Molecular Biology of the Cell by Alberts
- Molecular Cell Biology by Baltimore and Lodish

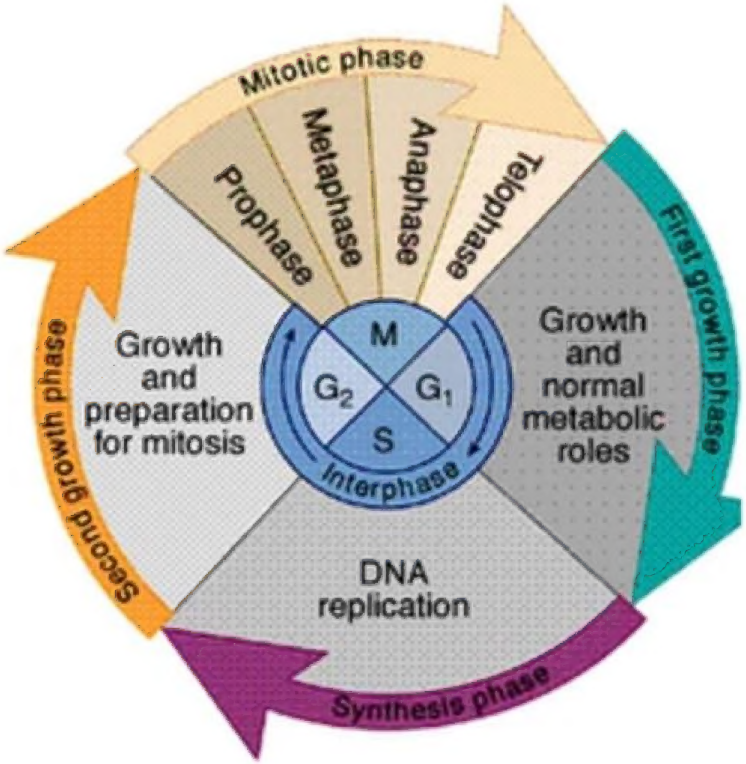
Cell Cycle

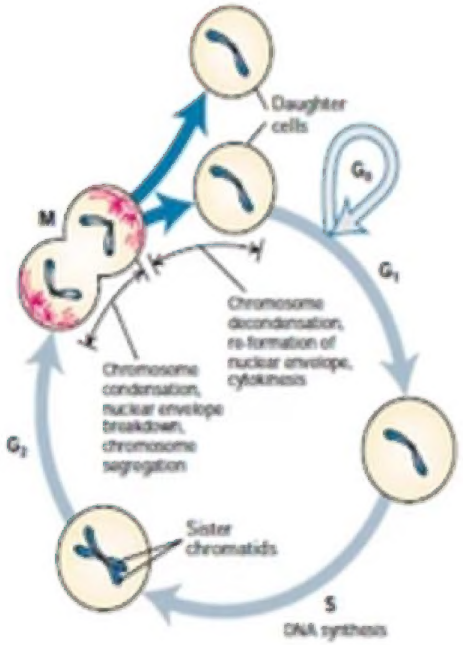
- To produce a pair of genetically identical cells, DNA must be perfectly replicated and the resulting chromosomes must be segregated into two separate cells.
- Most cells must also double their mass and duplicate their cytoplasmic organelles. To accomplish these goals, a cell must go through the Cell Cycle.
- A eukaryotic cell cannot divide into two, the two into four, etc. unless two processes alternate: **doubling** of its genome (**DNA**) in **S phase** (synthesis phase) of the cell cycle; **halving** of that genome during mitosis (**M phase**).
- The period between M and S is called $\mathbf{G_1}$; that between S and M is **G2**
- When a cell is in any phase of the cell cycle other than mitosis, it is often said to be in interphase.

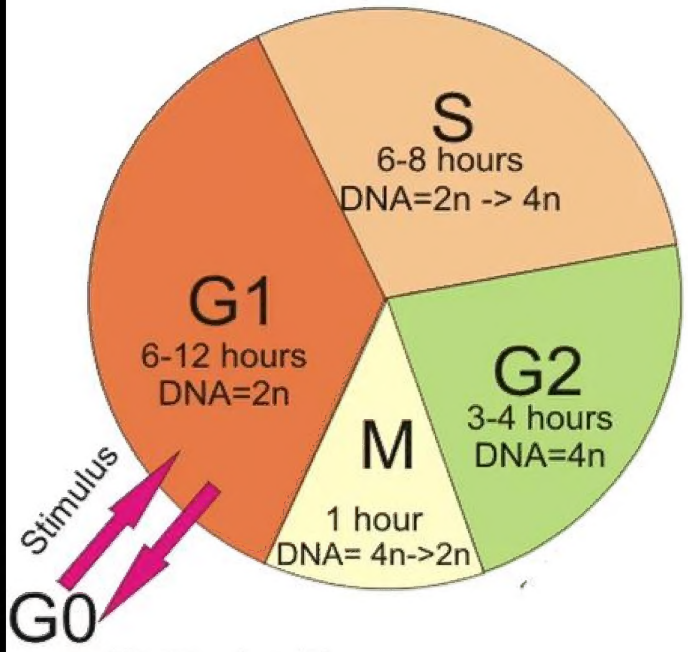
Cell Cycle

- Cells, such as nerve cells, muscle cells, or red blood cells, that are highly specialized and lack the ability to divide
- Cells that normally do not divide but can be induced to begin DNA synthesis and divide when given an appropriate stimulus- liver cells, lymphocytes
- Cells that normally possess a relatively high level of mitotic activity- hematopoietic stem cells

Phases of typical eukaryotic cell cycle



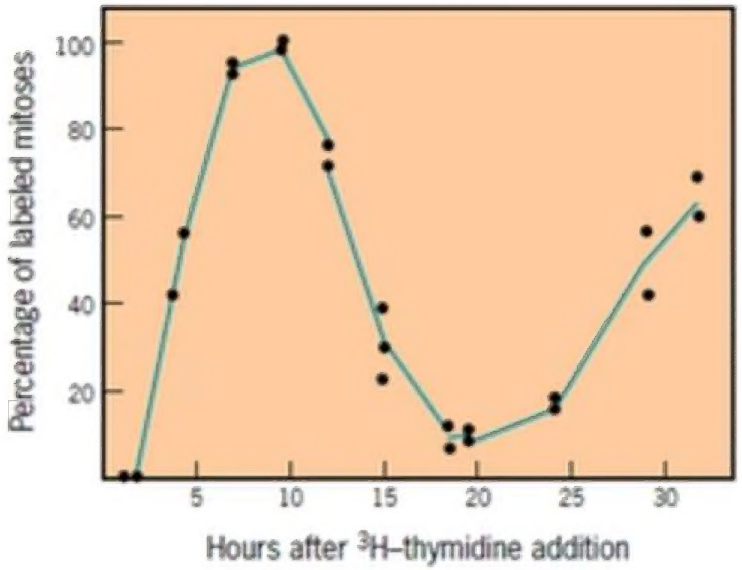




G0: Resting Phase
G1: Growth & Metabolism
S: DNA Replication
G2: Growth of Structural Elements
M: Mitosis

TABLE 18–1 SOME EUKARYOTIC CELL-CYCLE TIMES

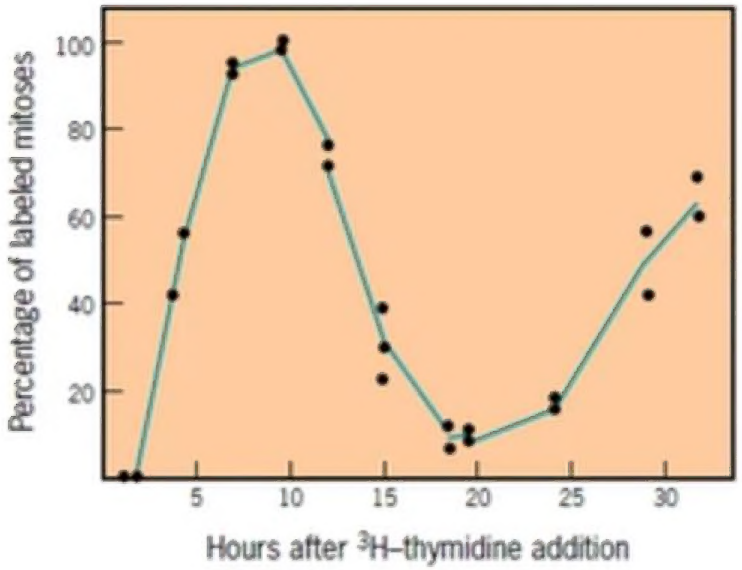
Cell Type	Cell-Cycle Times
Early frog embryo cells	30 minutes
Yeast cells	1.5 hours
Mammalian intestinal epithelial cells	~12 hours
Mammalian fibroblasts in culture	~20 hours



M phase and cytokinesis

HeLa cells were cultured for 30 minutes in medium containing [^{3}H]thymidine and then incubated for various times in unlabeled medium before being fixed and prepared for autoradiography. Each culture dish was scanned for cells that were in mitosis at the time they were fixed, and the percentage of those mitotic cells whose chromosomes were labeled

Experimental results demonstrating that replication occurs during a defined period of the cell cycle.



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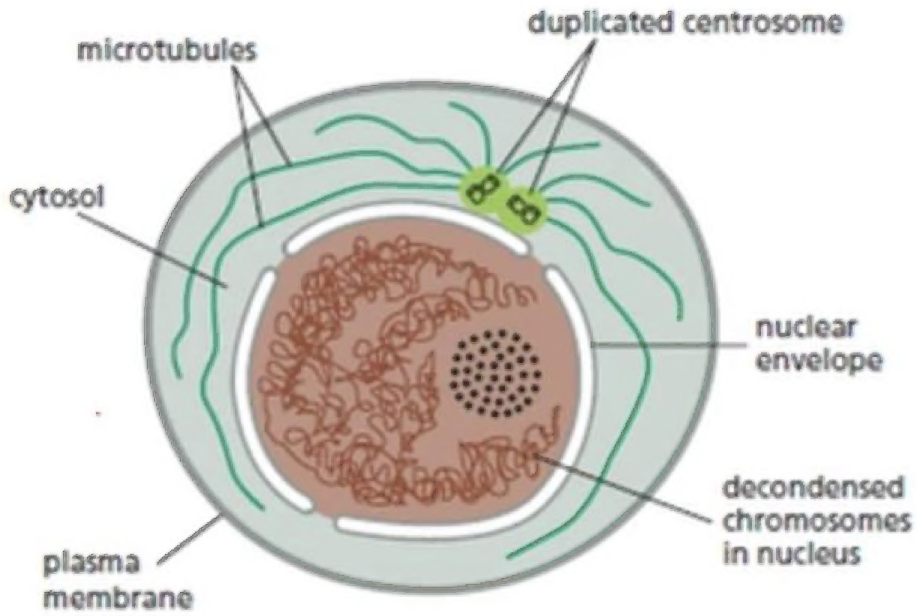
INTERPHASE



During interphase, the cell increases in size. The DNA of the chromosomes is replicated, and the centrosome is duplicated.

G1 phase

- G1 is the period of time between last mitotic division and beginning of synthesis of new DNA
- During G1, cell surveys the environment and make the decision to enter the cell cycle
- Proteins that promote cell division will be activated and force the cell towards the cell cycle

S phase

- The **S phase**, short for **synthesis phase**, is a period in the cell cycle during interphase, between G_1 phase and the G_2 phase.
- Following G_1, the cell enters the S stage, when DNA synthesis or replication occurs.
- At the beginning of the S stage, each chromosome is composed of one coiled DNA double helix molecule, which is called a chromatid.
- The enzyme DNA helicase splits the DNA double helix down the hydrogen bonds (the middle bonds). DNA polymerase follows, attaching a complementary base pair to the DNA strand, making two new semi-conservative strands.
- At the end of this stage, each chromosome has two identical DNA double helix molecules, and therefore is composed of two sister chromatids (joined at the centromere).
- During S phase, the centrosome is also duplicated. These two events are unconnected, but require many of the same factors to progress. The end result is the existence of duplicated genetic material in the cell, which will eventually be divided into two .

G2 phase

- The second growth phase of the cell cycle, consisting of the portion of interphase after DNA synthesis occurs

- A phase within interphase of the cell division cycle that prepares cells for mitosis

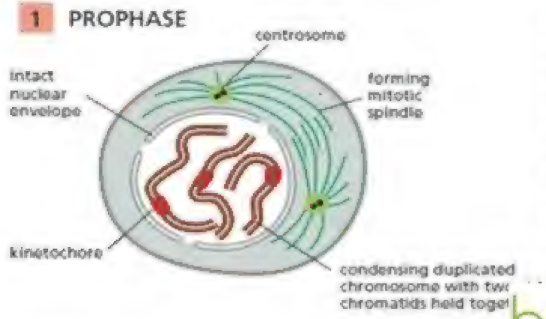
- Since the formation of new DNA is an energy draining process, the cell undergoes a second growth and energy acquisition stage, the G2 phase. The energy acquired during G2 is used in cell division

Mitosis or M Phase

- Cell growth and protein production stop at this stage in the cell cycle.
- All of the cell's energy is focused on the complex and orderly division into two similar daughter cells.
- Mitosis is much shorter than interphase, lasting perhaps only one to two hours.

Prophase

- During this first mitotic stage,
- The nucleolus fades and chromatin (replicated DNA and associated proteins) condenses into chromosomes.
- Each replicated chromosome comprises two chromatids, both with the same genetic information.
- Microtubules of the cytoskeleton, responsible for cell shape, motility and attachment to other cells during interphase, disassemble.
- The building blocks of these microtubules are used to grow the mitotic spindle from the region of the centrosomes.

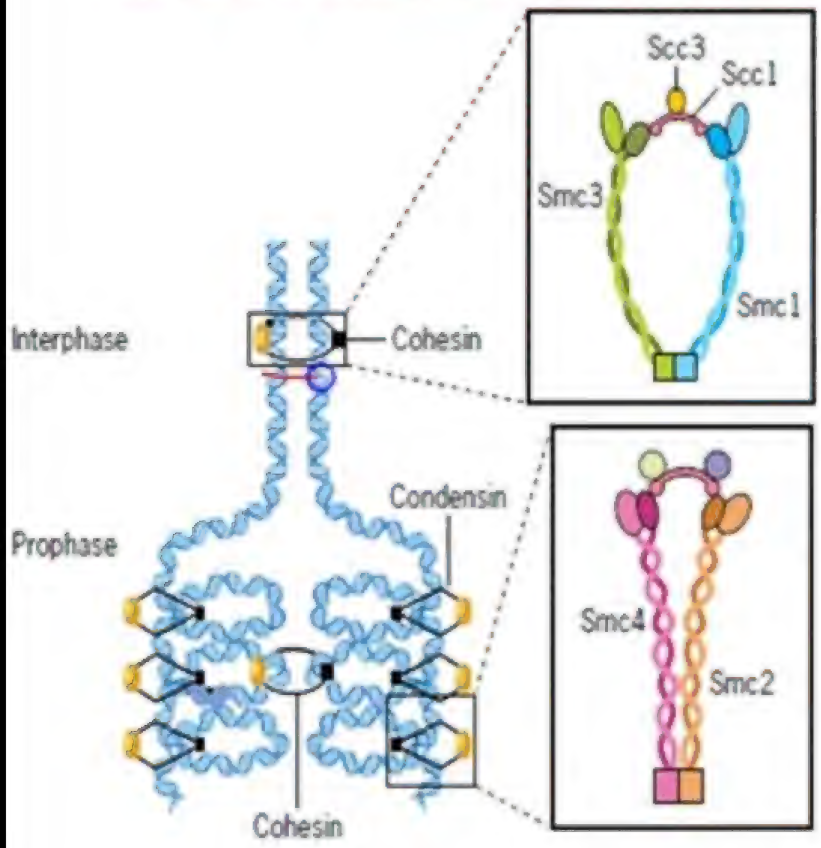


At prophase, the duplicated chromosomes, each consisting of two closely associated sister chromatids, condense. Outside the nucleus, the mitotic spindle assembles between the two centrosomes, which have begun to move apart. For simplicity, only three chromosomes are drawn.

Condensin

- Large protein complexes that play a central role in chromosome assembly and segregation during mitosis and meiosis.
- Condensins I and II share the same pair of core subunits, SMC2 and SMC4.
- Purified condensin I introduces positive superhelical tension into double-stranded DNA in an ATP-hydrolysis-dependent manner.
- Condensin II is present within the cell nucleus during interphase and is involved in an early stage of chromosome condensation within the prophase nucleus.
- On the other hand, condensin I is present in the cytoplasm during interphase, and gains access to chromosomes only after the nuclear envelope breaks down at the end of prophase.
- During prometaphase and metaphase, both condensin I and condensin II contribute to the assembly of condensed chromosomes
- Condensin is activated at the onset of mitosis by phosphorylation of several of its subunits by the cyclin– Cdk responsible for driving cells from G2 into mitosis

Model for the roles of condensin and cohesin in the formation of mitotic chromosomes



- condensin brings about chromosome compaction by forming a ring around supercoiled loops of DNA within chromatin.
- It is proposed that cooperative interactions between condensin molecules would then organize the supercoiled loops into larger coils, which are then folded into a mitotic chromosome fiber
- Both complexes are built around a pair of SMC subunits.
- Each of the SMC polypeptides folds back on itself to form a highly elongated antiparallel, coiled coil with an ATP-binding globular domain
- Cohesin and condensin also have two or three non-SMC subunits that complete the ring-like structure of these proteins.

Cohesin

- Protein complex that regulates the separation of sister chromatids during cell division, either mitosis or meiosis.
- Cohesins hold sister chromatids together after DNA replication until anaphase when removal of cohesin leads to separation of sister chromatids.
- Most of the cohesin dissociates from the arms of the chromosomes as they become compacted during prophase.
- Dissociation is induced by phosphorylation of cohesin subunits by two important mitotic enzymes called Polo-like kinase and Aurora B kinase
- Cohesin is thought to remain at the centromeres because of the presence there of a phosphatase that removes any phosphate groups added to the protein by the kinases

Kinetochore

- A complex protein structure called the kinetochore assembles at centromeres of each sister chromatid, attachment site for microtubules of mitotic spindle
- Interface between visible constriction in chromosome and microtubules of spindle
- One microtubule attaches to each centromere- yeast- point centromere
- Attach more microtubule to centromere- higher eukaryotes- regional centromere
- One kinetochore per chromatid

(1) the site of attachment of the chromosome to the dynamic microtubules of the mitotic spindle

(2) the residence of several motor proteins involved in chromosome motility

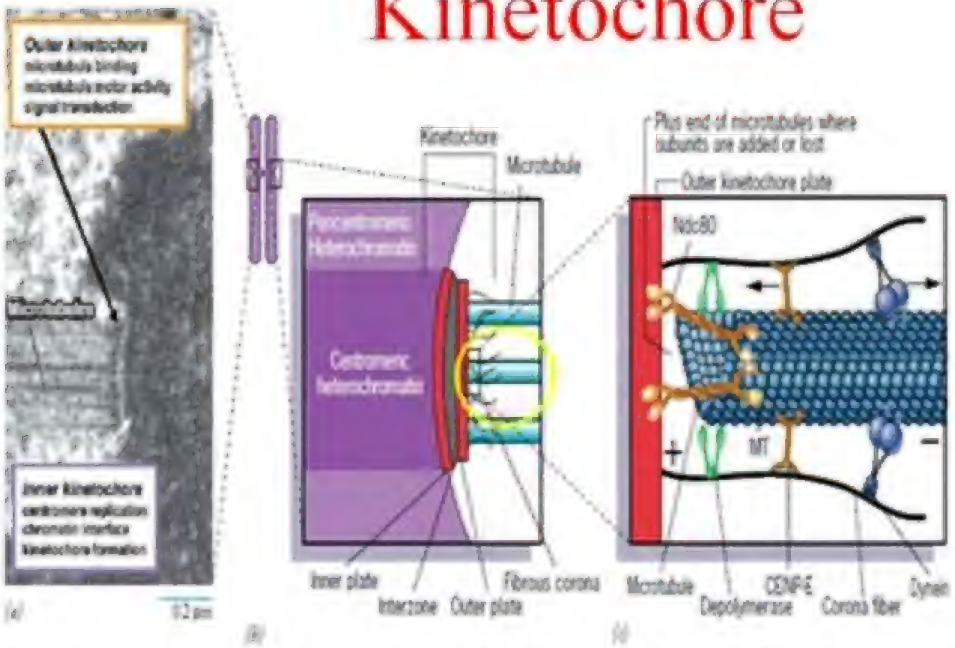
(3) a key component in the signaling pathway of an important mitotic Checkpoint

- motor proteins- CENP-E, which is a member of the kinesin superfamily and a rod-shaped protein called Ndc80- bind the surface of the adjacent microtubule.

Kinetochore

- Ndc80 is a protein complex consisting of four different protein subunits that forms a 50 nm-long, rod-shaped molecule extending outward from the body of the kinetochore.
- These Ndc80 fibrils have been implicated as couplers of the kinetochore to the plus end of a dynamic microtubule.

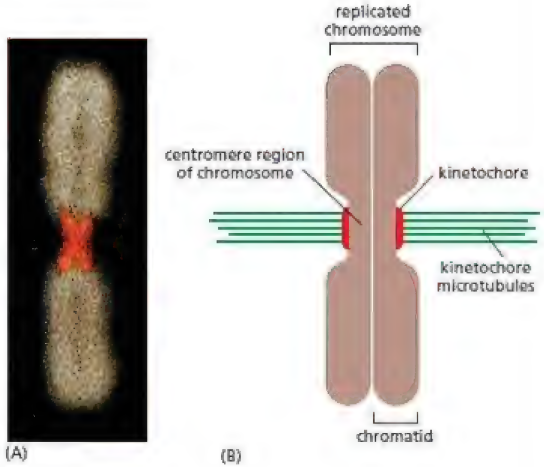
Kinetochore

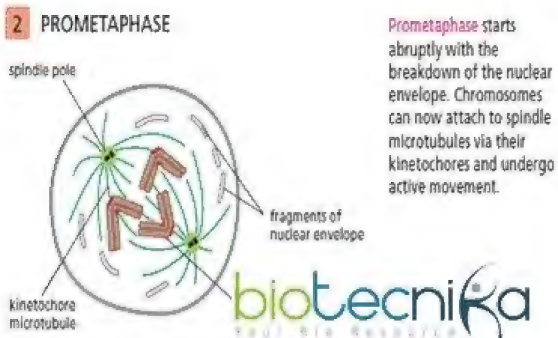


- Among the motor proteins associated with the kinetochore, cytoplasmic dynein moves toward the minus end of a microtubule, whereas CENP-E moves toward the plus end.
- These motors may also play a role in tethering the microtubule to the kinetochore.
- The protein labeled "depolymerase" is a member of the kinesin superfamily that functions in depolymerization of microtubules rather than motility. In this drawing, the depolymerases are in an inactive state (the microtubule is not depolymerizing).

Prometaphase

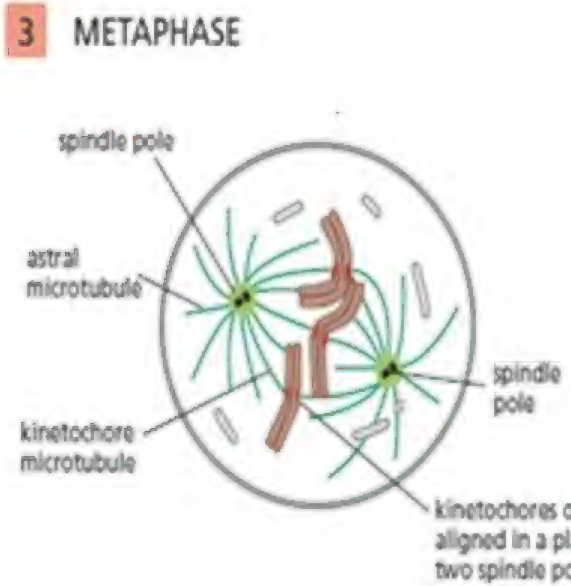
- The nuclear envelope breaks down so there is no longer a recognizable nucleus.
- Some mitotic spindle fibers elongate from the centrosomes and attach to kinetochores, protein bundles at the centromere region on the chromosomes where sister chromatids are joined.
- Other spindle fibers elongate but instead of attaching to chromosomes, overlap each other at the cell center.





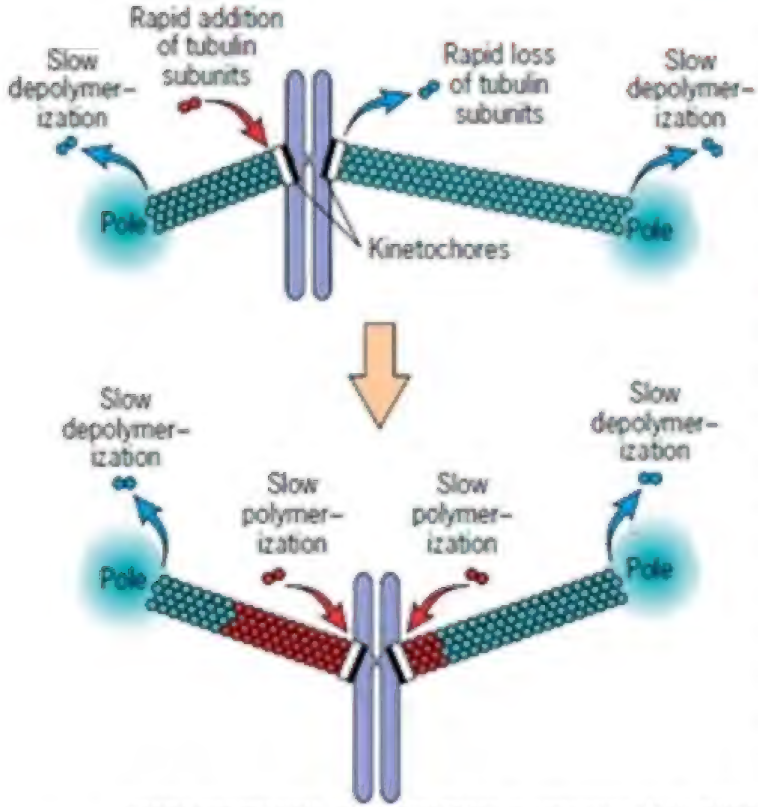
Metaphase

- Tension applied by the spindle fibers aligns all chromosomes in one plane (equitorial plate) at the center of the cell.
- Known as congression



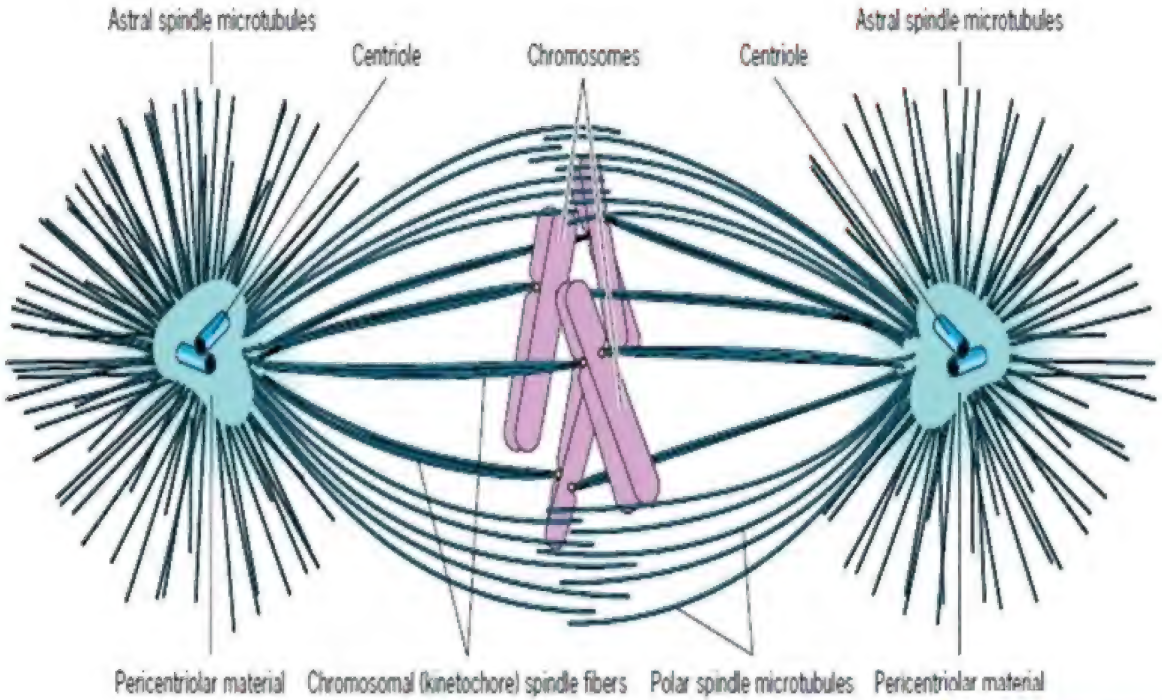
At metaphase, the chromosomes are aligned at the equator of the spindle, midway between the spindle poles. The kinetochore microtubules on each sister chromatid attach to opposite poles of the spindle.

Metaphase- Microtubule behavior during formation of the metaphase plate



- Initially, the chromosome is connected to microtubules from opposite poles that may be very different in length.
- As prometaphase continues, this imbalance is corrected as the result of the shortening of microtubules from one pole, due to the rapid loss of tubulin subunits at the kinetochore, and the lengthening of microtubules from the opposite pole, due to the rapid addition of tubulin subunits at the kinetochore.
- These changes are superimposed over a much slower polymerization and depolymerization that occur continually during prometaphase and metaphase, causing the subunits of the microtubule to move toward the poles in a process known as **flux**.

Metaphase



Metaphase

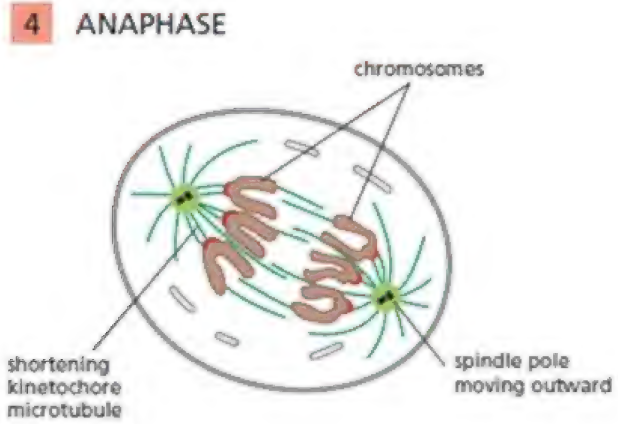
1. Astral microtubules that radiate outward from the centrosome into the region outside the body of the spindle. They help position the spindle apparatus in the cell and may help determine the plane of cytokinesis.
2. Chromosomal (or kinetochore) microtubules that extend between the centrosome and the kinetochores of the chromosomes. In mammalian cells, each kinetochore is attached to a bundle of 20–30 microtubules, which forms a spindle fiber. During metaphase, the chromosomal microtubules exert a pulling force on the kinetochores so are maintained in the equatorial plane by a "tug-of-war" between balanced pulling forces exerted by chromosomal spindle fibers from opposite poles.

During anaphase, chromosomal microtubules are required for the movement of the chromosomes toward the poles.

3. Polar (or interpolar) microtubules that extend from the centrosome past the chromosomes. Polar microtubules from one centrosome overlap with their counterparts from the opposite centrosome. The polar microtubules form a structural basket that maintains the mechanical integrity of the spindle.

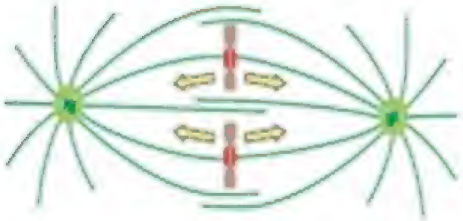
Anaphase

- Spindle fibers shorten, the kinetochores separate, and the chromatids (daughter chromosomes) are pulled apart and begin moving to the cell poles.
- Cohesin broken down

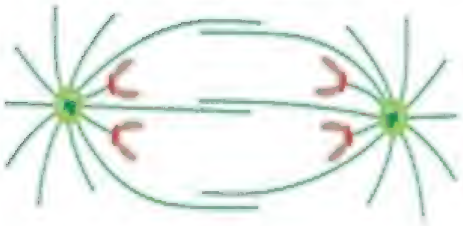


At anaphase, the sister chromatids synchronously separate and are pulled slowly toward the spindle pole to which they are attached. The kinetochore microtubules get shorter, and the spindle poles also move apart, both contributing to chromosome segregation.

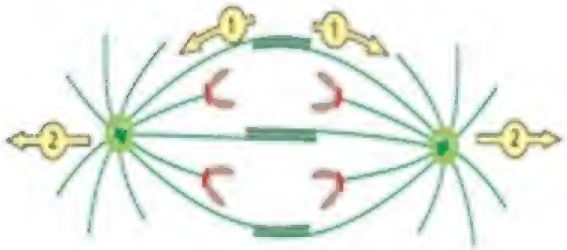
(A) ANAPHASE A CHROMOSOMES ARE PULLED POLEWARD



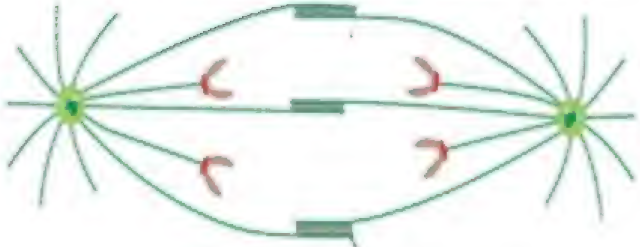
through a shortening of kinetochore microtubules, forces are generated at kinetochores to move chromosomes toward their spindle pole



(B) ANAPHASE B POLES ARE PUSHED AND PULLED APART



a sliding force (1) is generated between interpolar microtubules from opposite poles to push the poles apart; a pulling force (2) acts to pull the poles toward the cell cortex, thereby moving the two poles apart



microtubule growth at plus e

Securin

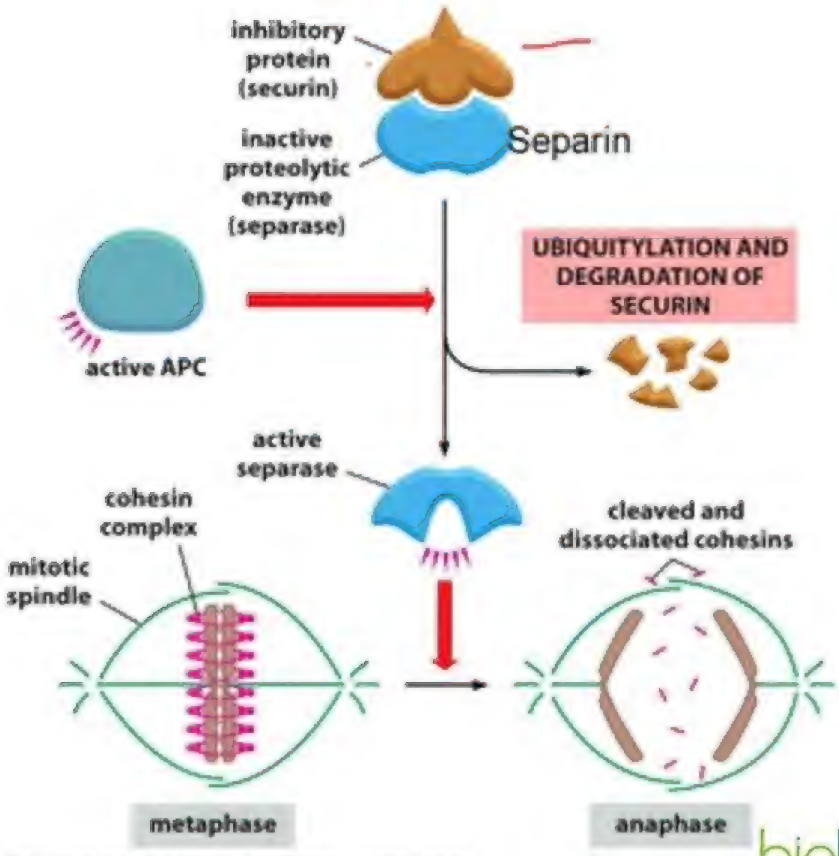
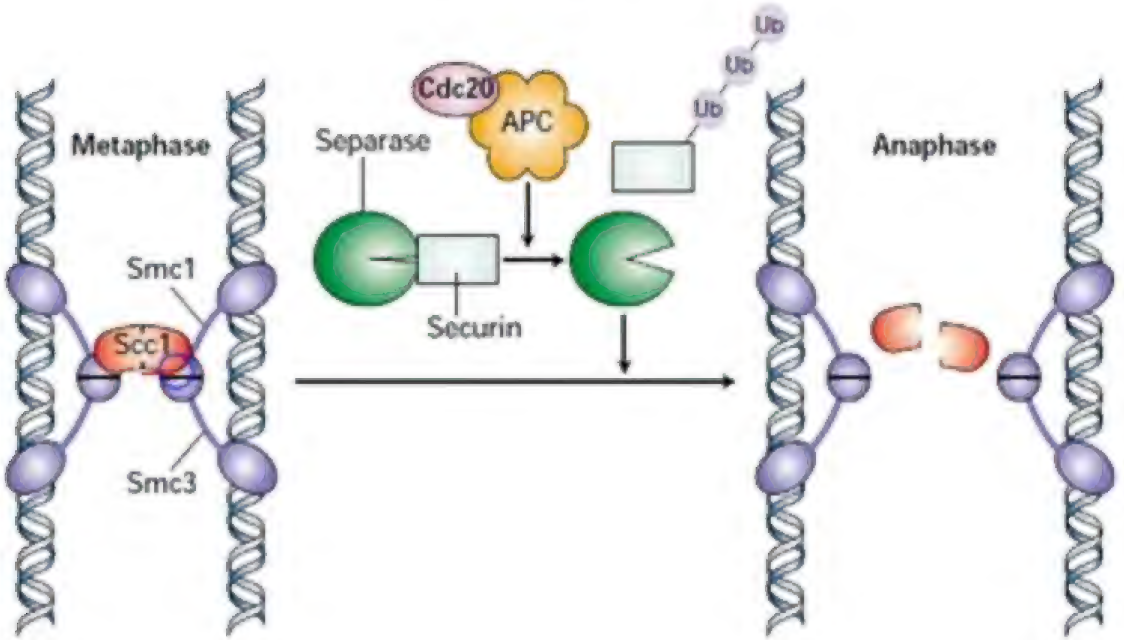
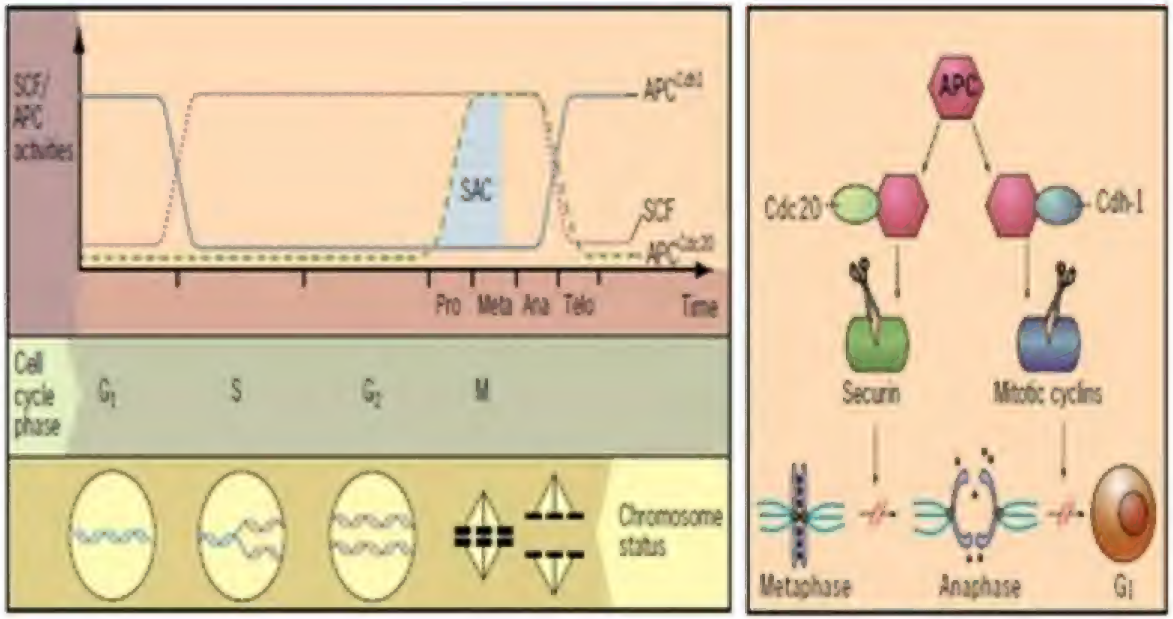


Figure 18-29 Essential Cell Biology 3/e (© Garland Science 2010)

SECURIN



SCF and APC activities during the cell cycle



(a) (b)

SCF and APC activities during the cell cycle

SCF and APC are multisubunit complexes that ubiquitinate substrates, leading to their destruction by proteasomes.

(a) SCF is active primarily during interphase, whereas APC (anaphase promoting complex) is active during mitosis and G1. Two different versions of APC are indicated. These two APCs differ in containing either a Cdc20 or a Cdh1 adaptor protein, which alters the substrates recognized by the APC. APCCdc20 is active earlier in mitosis than is APCCdh1. The label SAC stands for spindle assembly checkpoint. The SAC prevents APCCdc20 from triggering anaphase until all the chromosomes are properly aligned at the metaphase plate.

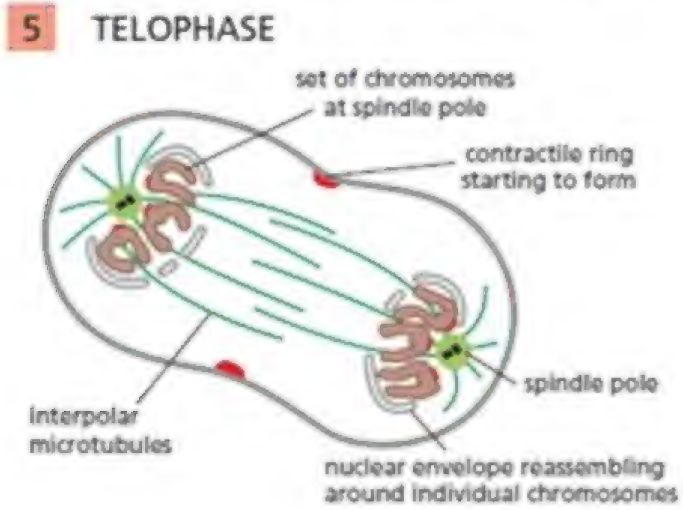
(b) APCCdc20 is responsible for destroying proteins, such as securin, that inhibit anaphase. Destruction of these substrates promotes the metaphase–anaphase transition. APCCdh1 is responsible for ubiquitinating proteins, such as mitotic cyclins, that inhibit exit from mitosis. Destruction of these substrates promotes the mitosis–G1 transition. APCCdh1 activity during early G1 helps maintain the low cyclin–Cdk activity required to assemble prereplication complexes at the origins of replication

Destruction of the cyclin by APC Cdh 1 leads to a precipitous drop in activity of the mitotic Cdk (cyclin B–Cdk1) and progression of the cell out of mitosis

phase of the next cell cycle

Telophase

- The daughter chromosomes arrive at the poles and the spindle fibers that have pulled them apart disappear.
- Nucleoli reappear
- New nuclear envelope
- Spindle breaks down into tubulin subunit



During telophase, the two sets of chromosomes arrive at the poles of the spindle. A new nuclear envelope reassembles around each set, completing the formation of two nuclei and marking the end of mitosis. The division of the cytoplasm begins with the assembly of the contractile ring.

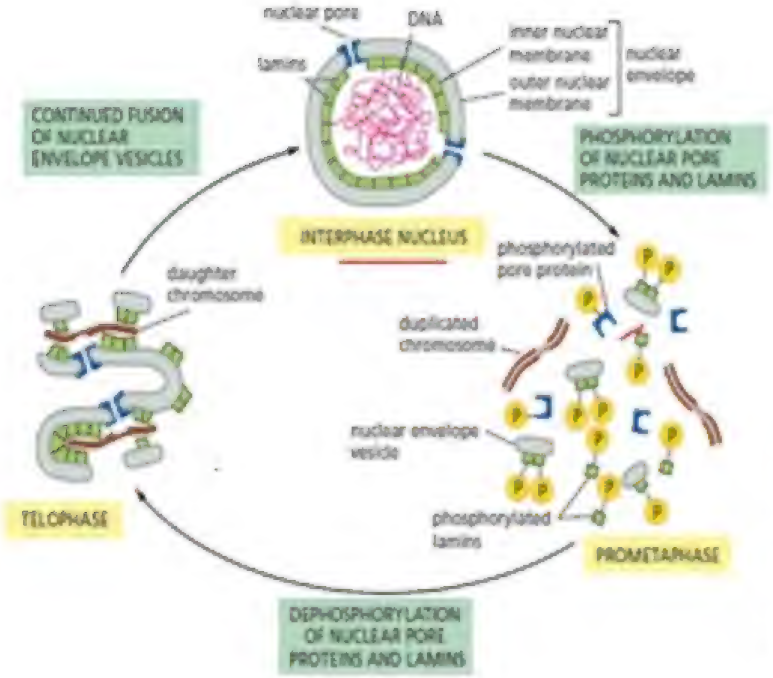


Figure 18-30 The nuclear envelope breaks down and re-forms during mitosis. The phosphorylation of nuclear pore proteins and lamins helps trigger the disassembly of the nuclear envelope at prometaphase. Dephosphorylation of these proteins at telophase helps reverse the process.

G0 Phase

- a cell is too small
- the cell is starved
- the environment does not provide the nutrients or energy needed for cell division

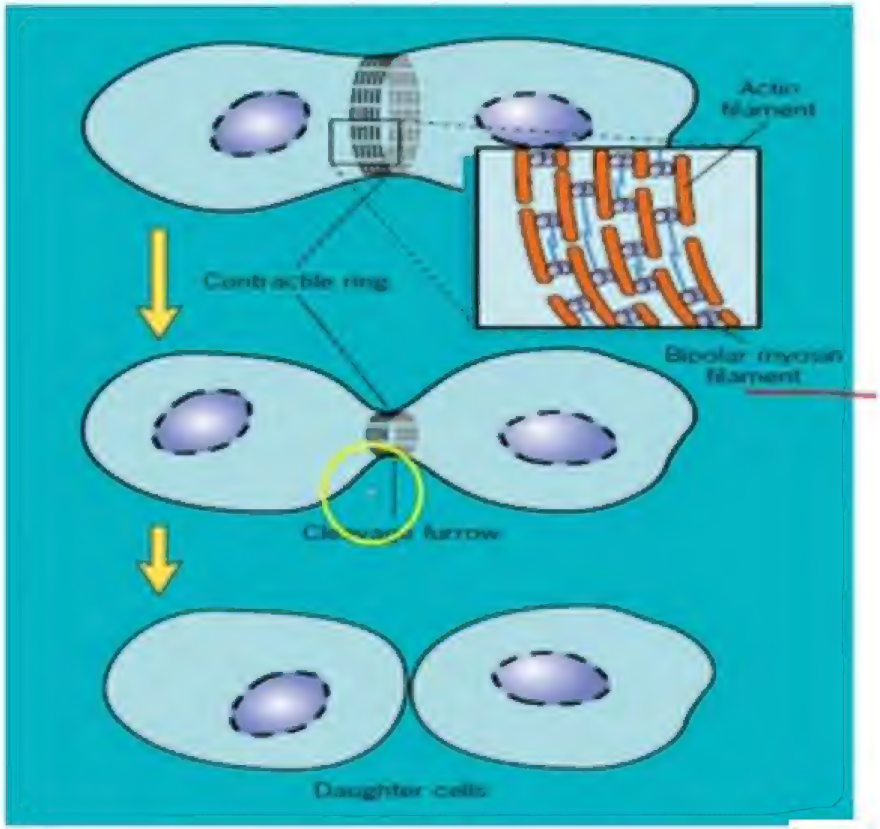
} Unfavorable circumstances for cell division

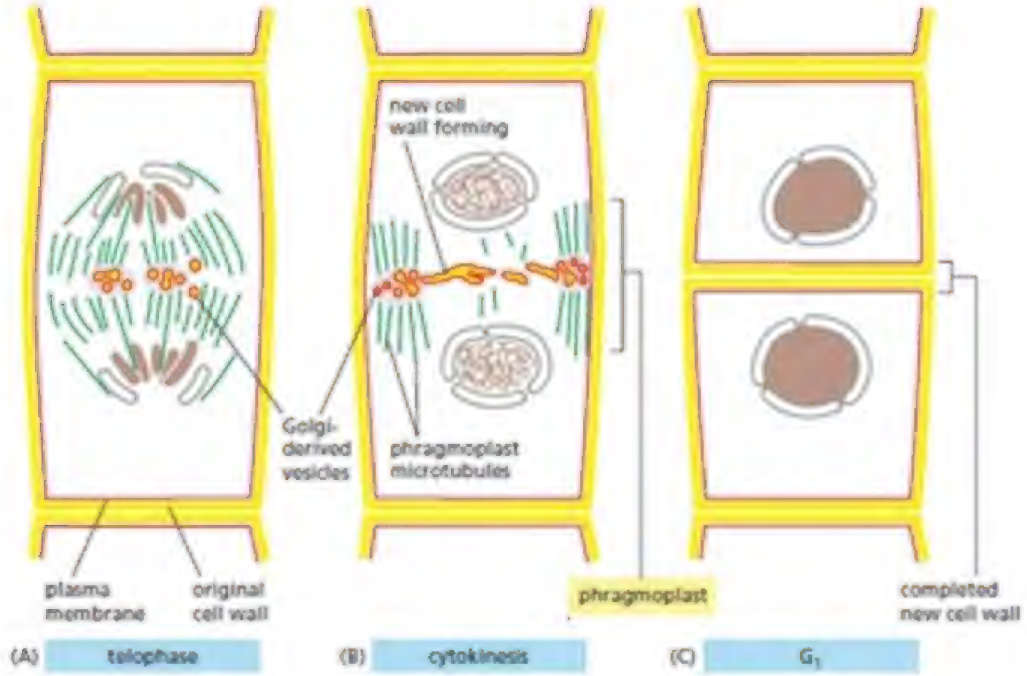
- Cells will halt and enter a phase called **"G-zero"** and the cells are said to be "Quiescent"
- During G0 phase, cells continue to perform their function vital for life albeit at a lower rate
- Cells in G0 accounts for the variability in the time between division in different tissue types
- **Eg.** Cells that line the intestine divide every 20 minutes
- Neurons and skeletal muscle rarely divides

Cytokinesis

- It is the division of cytoplasm. It occurs in animal cells by the appearance of a furrow in the middle of the cell. The furrow deepens and divides the cell into two. Microfilaments help in formation of furrow. Two daughter cells are formed
- The spindle fibers not attached to chromosomes begin breaking down until only that portion of overlap is left. It is in this region that a contractile ring cleaves the cell into two daughter cells.
- Microtubules then reorganize into a new cytoskeleton for the return to interphase.

Cytokinesis





- Phragmoplast attract golgi bodies- interpolar microtubules at the equator of the old mitotic spindle
- Golgi bodies forms vesicles filled with pectin
- Vesicles fuse to form a cell plate
- Contents of the vesicles form new middle lamella and cell walls of daughter cells while membrane form new cell surface mem

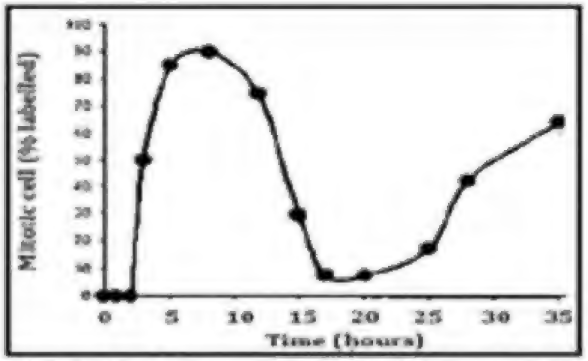
Facts

- Mitotic divisions without cytoplasmic divisions occur in many species to produce multinucleated or coenocytic cells
- Striated muscles
- Mycelia of mould
- Phloem of plants
- Mitosis in which spindle has centrioles and asters- astral or amphiastral- animal cells, lower plants- fungi, moss, fern, some algae
- Mitosis in which centrioles and asters are absent – anastral- higher plants

DIFFERENCES BETWEEN MITOSIS IN PLANT AND ANIMAL CELLS

MITOSIS IN ANIMAL CELLS	MITOSIS IN PLANT CELLS
1. Centrioles are involved	1. Centrioles are absent
2. Asters are formed	2. No aster formation
3. Cytokinesis occurs by furrowing of cytoplasm	3. Cytokinesis occurs by cell plate formation

80. In a given experiment the cells were labeled for 30 minutes with radioactive thymidine. The medium was then replaced with that containing unlabelled thymidine and the cells were grown for additional time. At different time points after replacement of medium the fraction of mitotic cells were analysed. Based on the results obtained, the above figure was drawn which shows the percentage of mitotic cells that are labeled as a function of time after brief incubation with radioactive thymidine.



Considering the above experiment, the following statemer were made:
A. Cells in the S-phase of the cell cycle during the 30 min labeling period contain radioactive DNA.
B. It takes about 3 hours before the first labeled mitotic ce appear.
C. The cells enter the second round of mitosis at t_{30} hours.
D. The total length of the cell cycle is about 27 hours with being more than 15 hours.

Which of the combination of above statements is correct?

(1) A and B (2) B and C
(3) C and D (4) A and D

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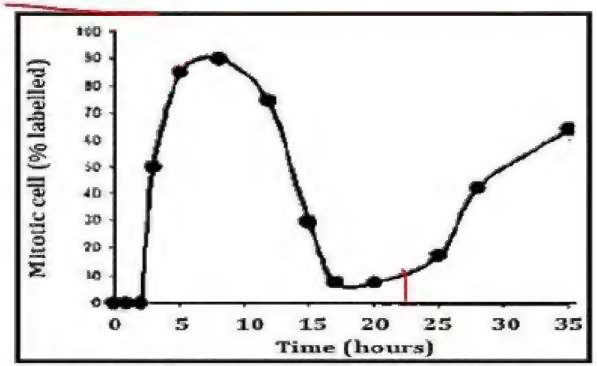
449 messages from 37 chats

drawn which shows the percentage of mitotic cells that are labeled as a function of time after brief incubation with radioactive thymidine.



Considering the above experiment, the following statemer were made:

A. Cells in the S-phase of the cell cycle during the 30 mint labeling period contain radioactive DNA.

B. It takes about 3 hours before the first labeled mitotic ce appear.

C. The cells enter the second round of mitosis at t_{30} hours.

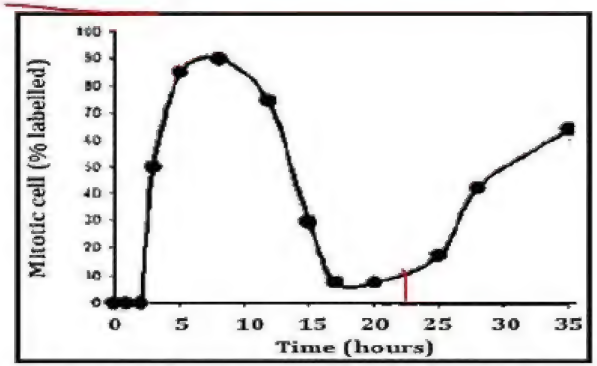
D. The total length of the cell cycle is about 27 hours with being more than 15 hours.

Which of the combination of above statements is correct?

(1) A and B (2) B and C

(3) C and D (4) A and D

80. In a given experiment the cells were labeled for 30 minutes with radioactive thymidine. The medium was then replaced with that containing unlabelled thymidine and the cells were grown for additional time. At different time points after replacement of medium the fraction of mitotic cells were analysed. Based on the results obtained, the above figure was drawn which shows the percentage of mitotic cells that are labeled as a function of time after brief incubation with radioactive thymidine.



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(1) A and B (2) B and C
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In an asynchronous culture the percentage of cells engaged in a particular activity is an approximate measure of the percentage of time that this activity occupies in the lives of the cell. An asynchronous culture of Hela cells is given a brief pulse with [3H] thymidine. If nuclei of 5% of cells is radioactively labeled and length of the complete cell cycle is 24 h then the length of the S phase can be considered approximately as

(1) 100 min(2) 120 min
(3) 60 min(4) 72 min

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Ans 4

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(3) 60 min(4) 72 min

$24 \times 60 = 1440$

$\frac{5 \times 1440}{100} = 72$ min

Ans 4

Reference

- Cell and Molecular Biology: concepts and experiments by Gerald Karp
- Molecular Biology of the Cell by Alberts
- Molecular Cell Biology by Lodish
- https://oncogenesandcancer.wordpress.com/cell-cycle-checkpoints-and-effect-of-oncogenes-2/

CELLULAR ORGANIZATION
Unit-2 D
Cell division and cell cycle

Batch- Morning

Duration : 1.5 Hrs.

Reference

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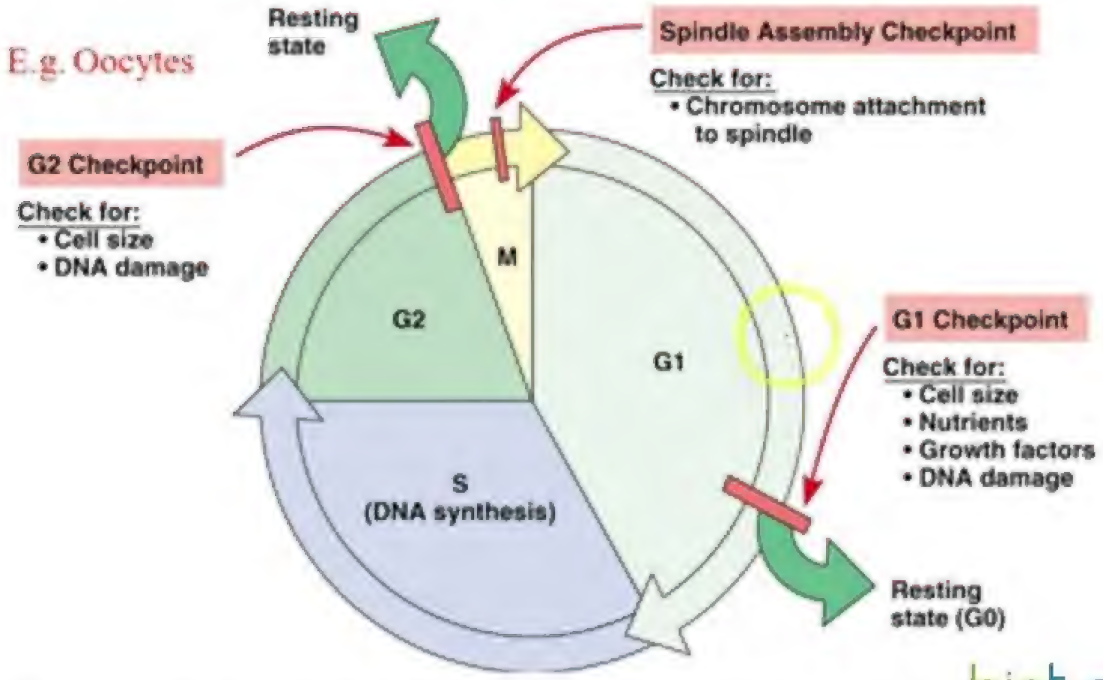
Cell Cycle Checkpoints

- Are control mechanisms that ensure the fidelity of cell division in eukaryotic cells.
- They verify whether the processes at each phase of the cell cycle have been accurately completed before progression into the next phase
- The checkpoints are surveillance mechanism and quality control of the genome to maintain genomic integrity.
- Checkpoint failure often causes mutations and genomic arrangements resulting in genetic instability. Genetic instability is a major factor of birth defects and in the development of many diseases, most notably cancer

Regulation of the Cell Cycle: Cell Cycle Checkpoints



Cell Cycle Checkpoints

- There are 3 checkpoints that take place in the cell cycle to prevent the cell from progressing to the next stage when it is not allowed. These checkpoints include; G1, G2 and a spindle checkpoint in Mitosis
- Arrest in the cell cycle is caused in the G1 and G2 phase by the P53 protein (which is a tumor suppressor gene).
- P53which is a transcription factor that then stimulates the expression of P21. P21 is a Cyclin-Kinase inhibitor that binds to and inhibits all Cdk-Cyclin complexes. This causes arrest in G1 and G2 phase. If the damage to the genome is extensive and cannot be repaired, P53 can also activate genes that code of apoptosis, or cell death.
- The spindle assembly checkpoint checks for whether the prerequisites have been met for chromosomes segregation, and therefore determines whether the chromosome segregation should take place or be delayed.
- This check point also arrests mitosis if the chromosomes are not aligned on the mitotic spindle and therefore will not allow for the proper attachment of spindle fibers on each sister chromatids and equal distribution of chromosomes to each daughter cell.

Cell Cycle Regulation

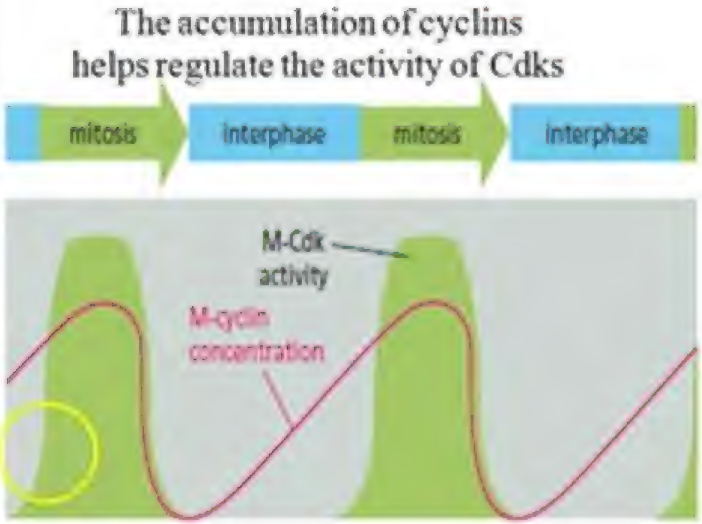
- Cell division is a vital process that requires orderly progression .
- The Cell Cycle is Regulated by Protein Kinases.
- Protein kinases are enzymes that phosphorylate proteins using ATP.
- A group of highly conserved serine/threonine kinases called cyclin-dependent kinases (CDKs) has been found to play a key role in guiding the cell cycle process.
- The regulated activity of CDKs is essential for the transitions from G1 to S and from G2 to M, and for the entry of nondividing cells into the cell cycle.
- The transition from G1 to S requires a set of cyclins different from those required in the transition from G2 to mitosis, where mitotic cyclins activate the CDKs .
- CDKs possess two tyrosine phosphorylation sites: One causes activation of the enzyme; the other causes inactivation.

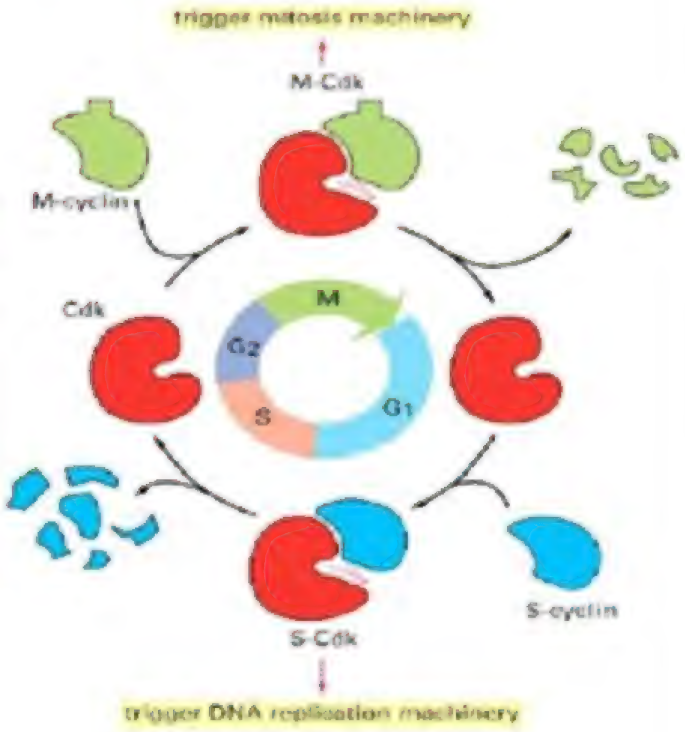
Cell Cycle Regulation

- G2 Checkpoint Control by MPF
- Entry of a cell into M phase is initiated by a protein called maturation promoting factor (MPF).
- MPF consists of two subunits: (1) a subunit with kinase activity that transfers phosphate groups from ATP to specific serine and threonine residues of specific protein substrates

  (2) a regulatory subunit called cyclin. The term cyclin was coined because the concentration of this regulatory protein rises and falls in a predictable pattern with each cell cycle

Cell Cycle Regulation





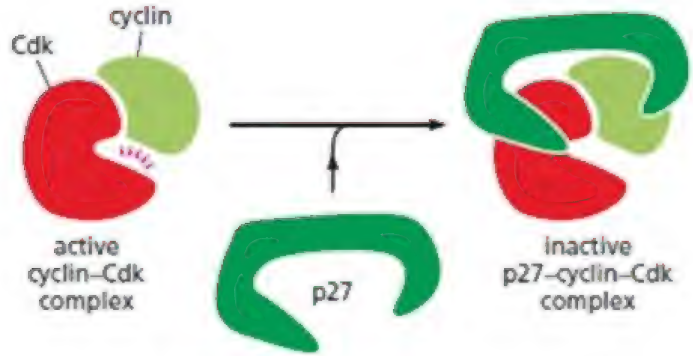
- CDK activity can be regulated in various ways, but two of the most important mechanisms are

  (1) cyclin synthesis and destruction - When a cyclin is present in the cell, it binds to the catalytic subunit of the Cdk, causing a major change in the conformation of the catalytic subunit

  (2)the phosphorylation and dephosphorylation of key amino acid residues within the CDK protein.

- At the G1-to-S transition, it uses Cdk inhibitors to keep cells from entering S phase and replicating their DNA
- At the G2-to-M transition, it suppresses the activation of M-Cdk by inhibiting the phosphatase required to activate the Cdk.
- And it can delay the exit from mitosis by inhibiting the activation of APC, thus preventing the degradation of M cyclin

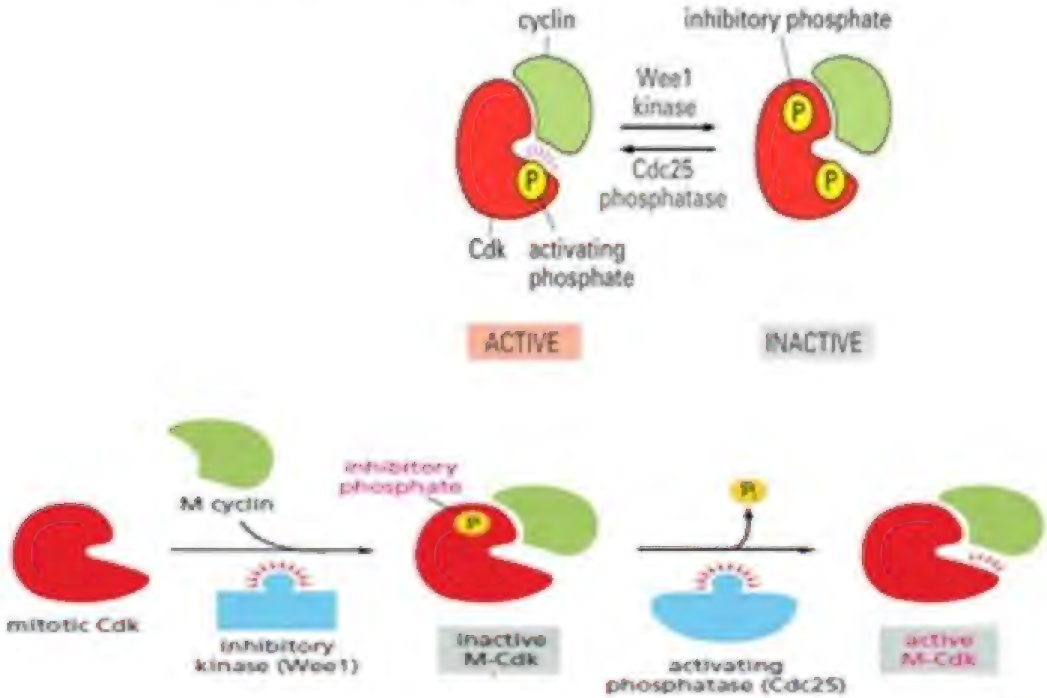
The activity of a Cdk can be blocked by the binding of a Cdk inhibitor



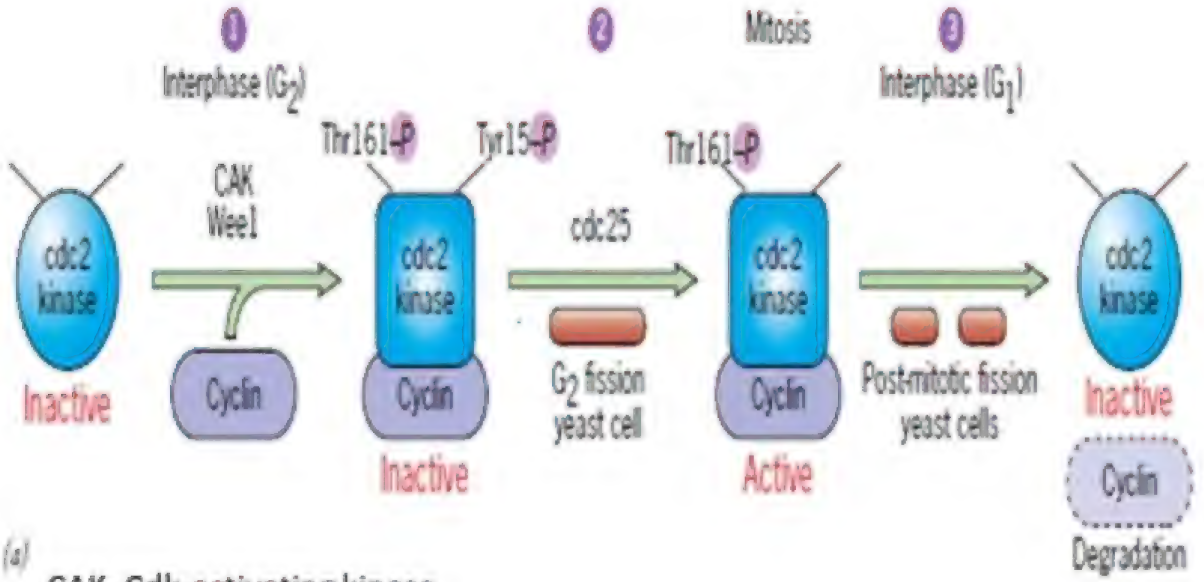
- the inhibitor protein (called p27) binds to an activated cyclin–Cdk complex. Its attachment prevents the Cdk from phosphorylating target proteins required for progress through G1 into S phase.

Cell Cycle Regulation by inhibiting phosphatase

In addition to cyclin activation, other layers of regulation are imparted by phosphorylation/dephosphorylation



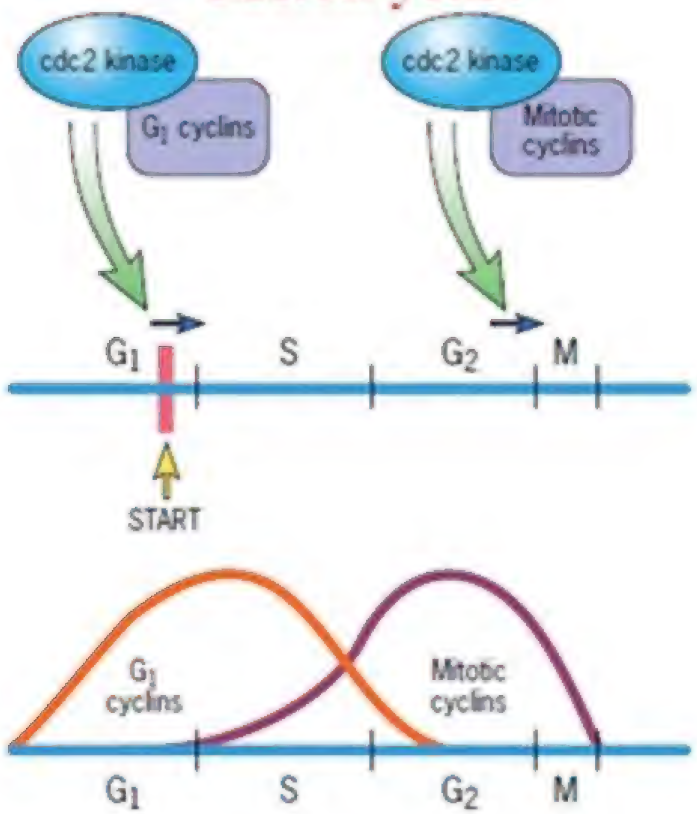
Regulation of Cdk1 in yeast cell cycle



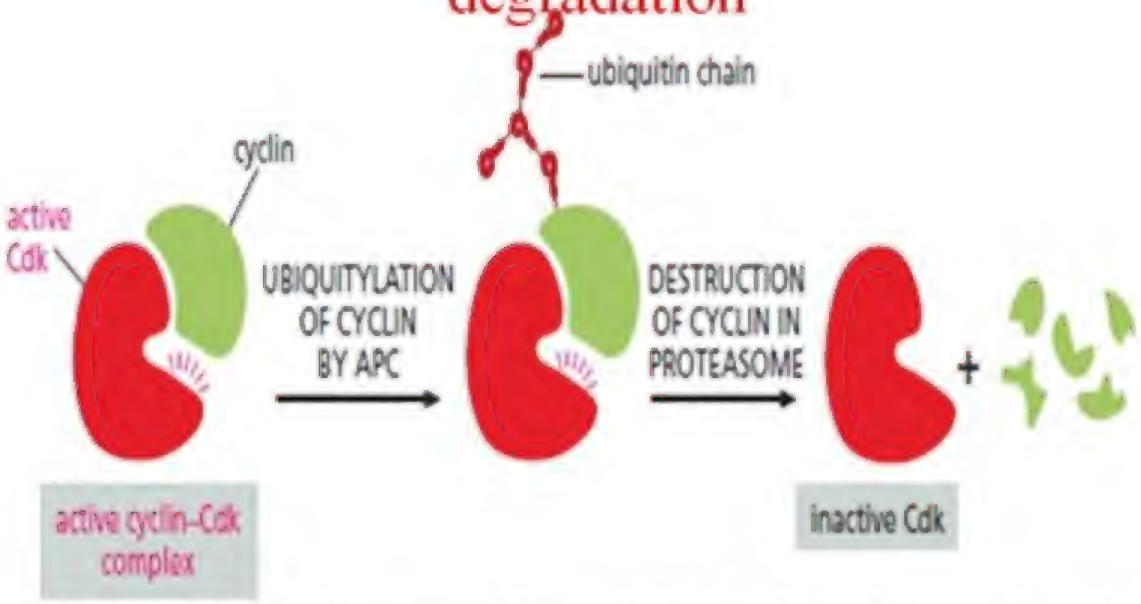
(a)

CAK- Cdk-activating kinase
CDK-Cyclin-dependent protein kinases
Cdc- cell-division cycle protein

A simplified model for cell cycle regulation in fission yeast



Activity of some Cdks is regulated by cyclin degradation



- Ubiquitylation of S or M cyclin by APC marks the protein for destruction in proteasomes.
- The loss of cyclin renders its Cdk partner inactive.
- Cyclin destruction can help drive the transition from one phase of the cell cycle to the next.

For example, M-cyclin degradation and the resulting inactivation of M-Cdk leads to the molecular events that take the cell out of mitosis

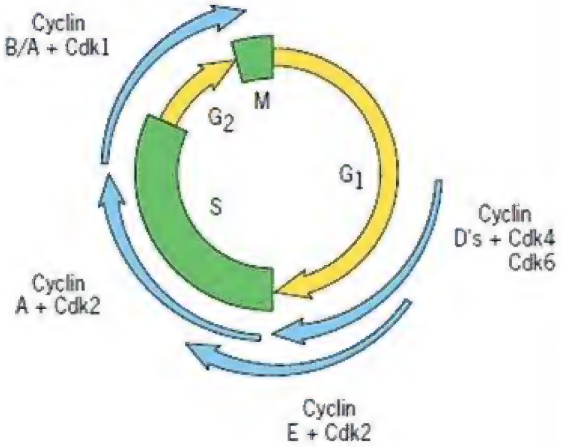
- Cyclin– Cdk complex contains inhibitory phosphates, and to become active. the Cdk must be dephosphorylated by a specific protein phosphatase

Cell Cycle Regulation

TABLE 18–2 THE MAJOR CYCLINS AND CDKS OF VERTEBRATES

Cyclin–Cdk Complex	Cyclin	Cdk Partner
G_1-Cdk	cyclin D*	Cdk4, Cdk6
G_1/S-Cdk	cyclin E	Cdk2
S-Cdk	cyclin A	Cdk2
M-Cdk	cyclin B	Cdk1

*There are three D cyclins in mammals (cyclins D1, D2, and D3).



Four classes of cyclins

1. G1-cyclins — help to promote passage through " Start" or the restriction point in late G1

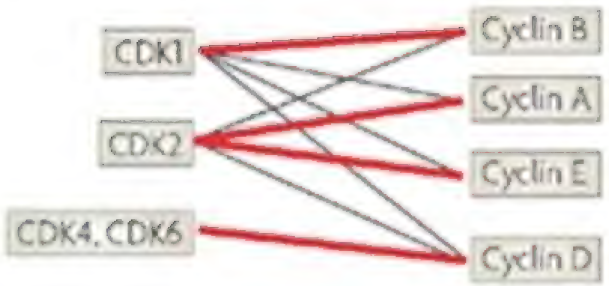
2. G1/S-cyclins — bind Cdks at the end of G1 and commit the cell to DNA replication

3. S-cyclins – binds Cdks during S phase and are required for the initiation of DNA replication

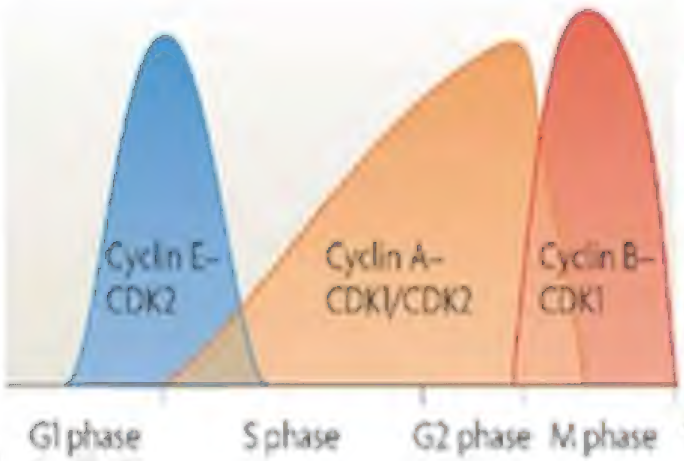
4. M-cyclins — promote the events of mitosis

b) CDK1 and CDK2 bind to multiple cyclins (cyclin types A, B, D and E), whereas CDK4 and CDK6 only partner D-type cyclins.
Thick lines represent the preferred pairing for each kinase.

b

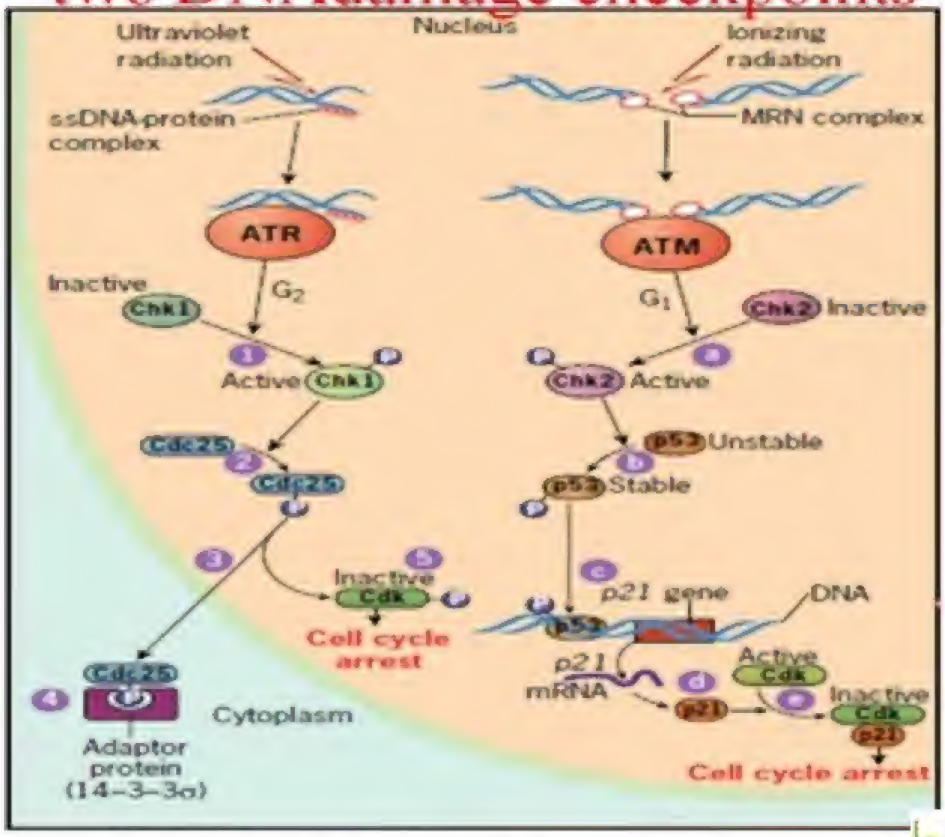


c



c) According to the classical model of cell cycle control, D-type cyclins and CDK4 or CDK6 regulate events in early G1 phase (not shown), cyclin E-CDK2 triggers S phase, cyclin A-CDK2 and cyclin A-CDK1 regulate the completion of S phase, and CDK1-cyclin B is responsible for mit

Models for the mechanism of action of two DNAdamage checkpoints

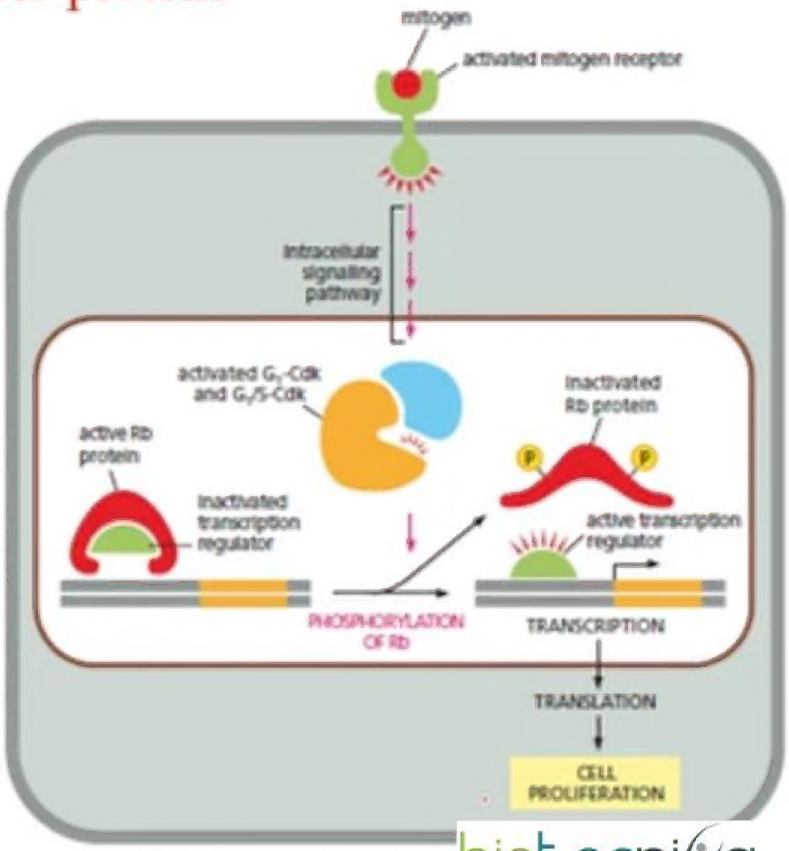


Models for the mechanism of action of two DNA damage checkpoints

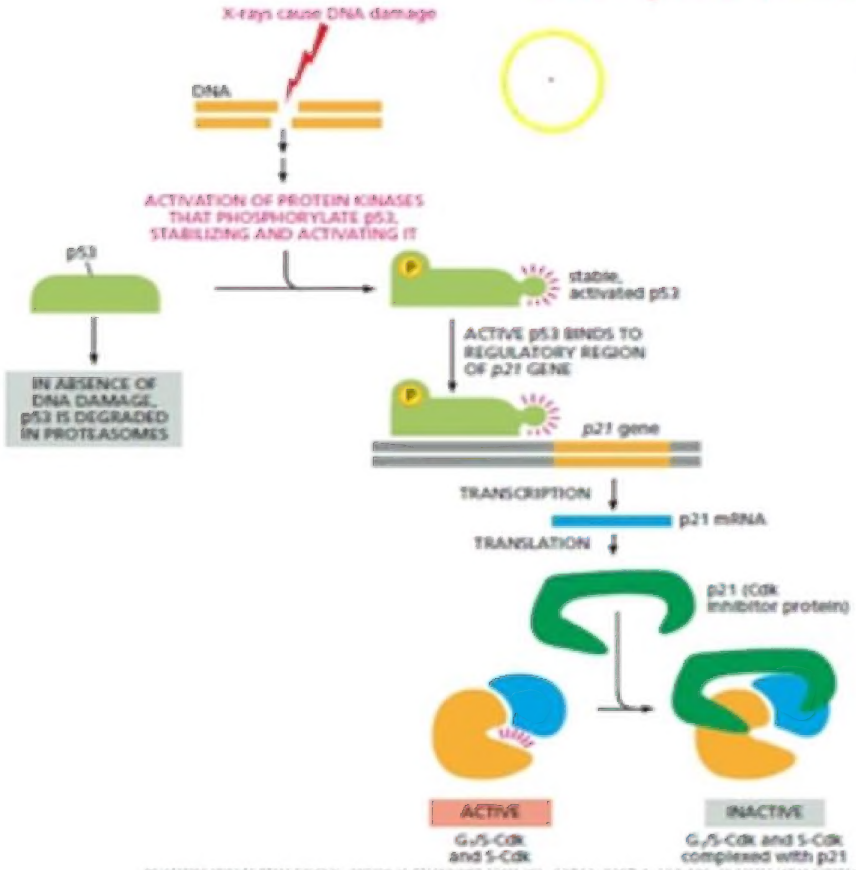
- ATM and ATR are protein kinases that become activated following specific types of DNA damage.
- ATM becomes activated in response to double-strand breaks, which are detected by the MRN protein complex. ATR, on the other hand, becomes activated by protein-coated ssDNA that forms when replication forks become stalled or the DNA is being repaired after various types of damage. In the G2 pathway, ATR phosphorylates and activates the checkpoint kinase Chk1 (step 1), which phosphorylates and inactivates the phosphatase Cdc25 (step 2), which normally shuttles between the nucleus and cytoplasm (step 3).
- Once phosphorylated, Cdc25 is bound by an adaptor protein in the cytoplasm (step 4) and cannot be reimported into the nucleus, which leaves the Cdk in its inactivated, phosphorylated state (step 5).
- In the G1 pathway, ATM phosphorylates and activates the checkpoint kinase Chk2 (step a), which phosphorylates p53 (step b). p53 is normally very short-lived, but phosphorylation by Chk2 stabilizes the protein, enhancing its ability to activate *p21 transcription (step c). Once transcribed and* translated (step d), p21 directly inhibits the Cdk (step e).

Mitogens stimulate cell proliferation is by inhibiting the Rb protein

- In the absence of mitogens, dephosphorylated Rb protein holds specific transcription regulators in an inactive state; these transcription regulators are required to stimulate the transcription of target genes that encode proteins needed for cell proliferation.
- Mitogens binding to cell-surface receptors activate intracellular signaling pathways that lead to the formation and activation of G1-Cdk and G1/S-Cdk complexes.
- These complexes phosphorylate, and thereby inactivate, the Rb protein, releasing the transcription regulators that activate the transcription of genes required for cell proliferation.



DNA damage can arrest the cell cycle in G1



- When DNA is damaged, specific protein kinases respond by both activating the p53 protein and halting its normal rapid degradation.
- Activated p53 protein thus accumulates and stimulates the transcription of the gene that encodes the Cdk inhibitor protein p21.
- The p21 protein binds to G1/S-Cdk and S-Cdk and inactivates them, so that the cell cycle arrests in G1.

Overview of checkpoint controls in the cell cycle